Phycoerythrin Signatures in the Littoral Zone

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LONG-TERM GOALS

My long term goal is to understand how adaptive phenotypes evolve in marine organisms, especially marine phytoplankton. This research examines the role of the optical environment in the evolution of the light harvesting apparatus of marine picocyanobacteria, specifically those with phycoerythrin as their principal light harvesting pigment.

OBJECTIVES

Phycoerythrin (PE) refers to a family of highly fluorescent, water-soluble pigments that are the primary light harvesting pigments of many marine picocyanobacteria, a globally important group of marine phytoplankton. In marine *Synechoccocus* and many other picocyanobacteria, these pigments are typically organized into phycobilisomes, macromolecular structures that provide nearly all light energy for Photosystem II (Glazer, 1999). Thus, the wavelengths of light that can be used for photosynthesis are determined by the spectral forms of PE the organisms synthesize. This, in turn, depends on the relative concentration of two different chromophores that can be incorporated into the PE heterodimer. These are phycoerythrobilin (PEB, λ_{AbsMax}, ~550nm), found in all PEs, and phycourobilin (PUB, λ_{AbsMax}, ~500 nm) found in varying concentrations in some forms of PE. PEB provides for efficient utilization of green wavelengths of light; as the relative abundance of PUB increases, the spectral signature of the PE becomes more complex (i.e. additional peaks and shoulders in the fluorescence excitation and absorption spectrum). The ecological effect of increasing the PUB:PEB ratio is that the cell's ability to use shorter wavelengths that penetrate more transparent seawater greatly increases (Fig. 1).

We have found that PEs with different spectral signatures, resulting from differences in the PEB:PUB ratio tend to dominate in the water masses for which they show chromatic complementarity (Wood et al., 1998, 2003; Fig. 5 in Coble et al., 2004, and unpublished data from this project). In an effort to determine how this complementarity is achieved, we hypothesize that the PUB:PEB ratio of the PE studied in bulk seawater might track the available wavelengths if 1) there was a diversity of PE-containing picocyanobacterial genotypes in the water, each had different PUB:PEB ratios, and their relative abundance changed as the light field selected for strains with differing abilities to harvest blue and green light or 2) the PE-containing picocyanobacteria were able to chromatically adapt; that is, to change the PUB:PEB ratio of the PE in response to changes in the wavelength of available light. The first mechanism is essentially a population genetic or microevolutionary mechanism and would involve

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a change in the genetic composition of the picocyanobacterial community. The second is a physiological mechanism that would not require a change in community structure. As part of this research, we have isolated strains capable of chromatic adaptation (CA) and demonstrated that the biochemical mechanism of CA in these strains is a novel form of chromatic adaptation, previously unreported in cyanobacteria (Everroad & Six et al., 2006). However, we also found that these strains co-occurred in a diverse local community of strains with different PUB:PEB ratios, most of which were not capable of CA. Thus, it appears that either population genetic or physiological mechanisms, or both, might underlie the ability of PE-containing marine *Synechococcus* communities to track the spectral composition of the natural light field.

In the current reporting period, our primary objective were to further examine the genetic diversity of marine picophytoplankton in natural environments, correlate the spectral phenotype of the cells with their genotype, and use these data to help understand how spectral phenotype has evolved.

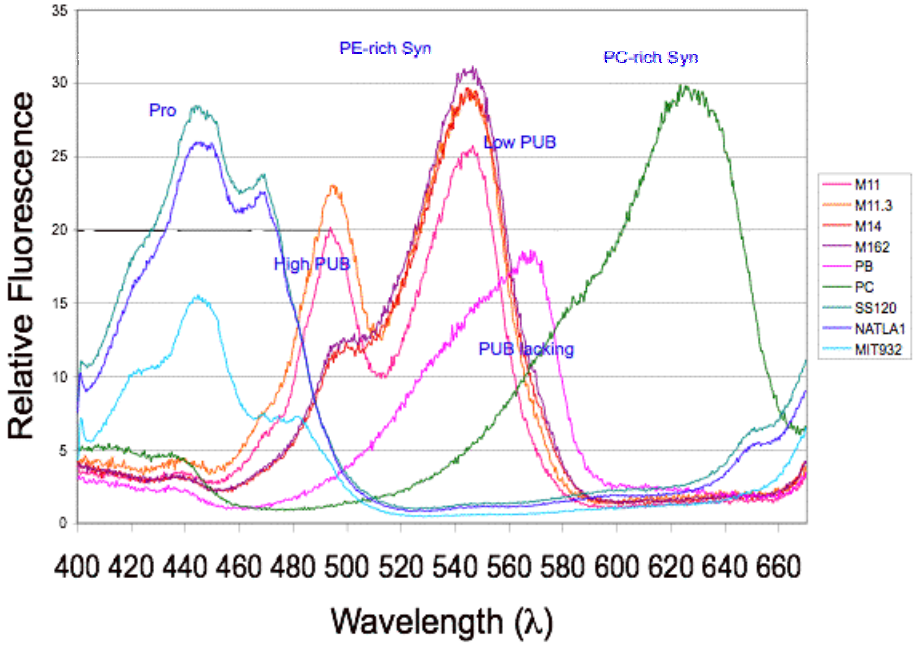

Figure 1. Excitation spectra for fluorescence emission from chlorophyll (680 nm) in whole cells of marine picocyanobacteria – roughly correlating with action spectra for oxygen evolution. Prochlorococcus relies primarily on blue wavelengths, phycoerythrin lacking (PC-rich) strains use primarily red light, and strains with phycoerythrin use primarily green light (PUB lacking) or a combination of green and bluegreen light (High and low PUB). These represent different spectral phenotypes and the great differences in wavelengths that can be used for photosynthesis highlights the likelihood that strains with different spectral phenotypes will perform very differently in different optical environments.

APPROACH

This work has involved a laboratory and a field component. Samples for recent work were collected in subtropical waters of the Gulf of Mexico, Florida Bay, and the Straights of Florida. This region was chosen because preliminary data showed picocyanobacteria to be very abundant (10^4-10^6 ml^{-1}) in these warm subtropical waters, and ocean color varies in this region. Clear oceanic waters interface with turbid organic rich waters and eutrophic waters with high chlorophyll, leading to a complex light field likely to maintain genetic diversity in spectral type. Genetic diversity of the picocyanobacterial community is estimated using culture-based and culture-free methods. For most samples, the picoplankton community is counted by flow cytometry and the spectral signature of bulk PE estimated by fluorescence spectroscopy (Li and Wood, 1988; Wood et al. 1985, 1999). Water samples are used to establish clonal isolates of picocyanobacteria, the spectral signature of the culture is determined by fluorescence spectroscopy and the spectral phenotype described as high, medium, low, or no PUB (Fig. 1). All strains are screened for the ability to chromatically adapt before being assigned to a category of fixed spectral phenotypes. Strains are genetically characterized using the sequence of three genetic markers obtained using PCR-based methods as described by Everroad and Wood (2006). Two of these are *rpo*C1 and 16S rDNA; these genes are traditionally used provide resolution of the phylogenetic history of prokaryotes as several levels or resolution. The third genetic marker we use are the co-transcribed genes for the apoproteins of PE (*cpe*BA and *mpe*BA); these are colorless proteins to which the PEB and PUB chromophores are attached. As such, they are an integral component of the genetic architecture of the spectral phenotype. Two forms of the PE apoprotein are known from marine *Synechococcus*: CpeBA, which forms the basis of Class I PEs (PEI), is found in all marine *Synechococcus*, and MpeBA, which forms the basis of Class II PEs, and is found only in some marine *Synechococcus*, (PEII; Ong and Glazer 1991, Glazer 1999). The apoproteins for these pigments are coded for by *cpe*BA and *mpe*BA genes, respectively. PCR primers for these genes were developed for this project (Everroad and Wood, 2006) and have successfully amplified cyanobacterial PEs from throughout the cyanobacterial radiation and from field samples in both our hands and others'.

Since even a successful culturing effort results in cultures from only a miniscule percentage of the original cells in a natural population, we know that culture-based methods underestimate diversity. Thus, in addition to genetically characterizing cultures from each water sample, water is filtered and DNA extracted in bulk from the picoplankton community on the filter. Sequences for 16S *r*DNA genes and genes for PE apoproteins from this environmental DNA are amplified using cyanobacterial specific primers for the small subunit (SSU) genes and our PE primers, providing insight into the variety of genes at these loci present in the natural population.

Standard phylogenetic methods of inference are used to examine the scale of diversity and relatedness of sequences obtained from environmental DNA samples and the new clonal isolates. Data from clonal cultures that have a well-characterized spectral phenotype is used to investigate the evolution of spectral phenotype. Comparison of the phylogeny provided by the housekeeping genes with that provide by the *cpe*BA and *mpe*BA genes is used to infer the evolution of various spectral phenotypes. Congruence of the phylogenies suggests that the spectral phenotypes evolve in each lineage of organisms by selective sweeps following mutation. Incongruence is more consistent with acquisition of the gene, and presumably the spectral phenotype it is associated with, by lateral gene transfer.

WORK COMPLETED

By far the majority of work done on the project this year was conducted by Craig Everrroad, the Ph.D. student supported on this project. Fieldwork and most laboratory work were completed last year. Most of this year was spent completing analysis for the final two chapters of his dissertation, one relating to the evolution of spectral phenotype and the other to evaluate the range of genetic diversity in a natural population of picocyanobacteria from the Gulf of Mexico. In addition, since earlier fieldwork has provided a rich store of frozen environmental samples from a range of optically diverse environments on the Florida shelf, he has worked with Allison Poole, a new master's student, to teach her methods needed to complete a thesis with these samples. Funds for reagents, sequencing, and a teaching fellowship are available from the University of Oregon to support her in this work. Their preliminary results show the presence of a number of novel sequences for both genes.

RESULTS

The most significant result from work conducted this year is the finding that the apoprotein genes for PE from strains that span a wide range of spectral phenotypes show a very different phylogenetic pattern than the housekeeping genes from the same strains. The 16S *r*DNA gene is widely used for phylogenetic analysis as is *rpoC1*; 16S *r*DNA sequence data allows us to determine the location of our study strains within the cyanobacterial radiation, while sequence data from the more rapidly evolving *rpoC1* allows us to resolve the shallower relationships between our strains and other closely related picocyanobacteria. These genes, taken together, provide a strong indication of phylogenetic history and identify strains with common ancestry. As noted earlier, we sequenced the *cpe*BA and *mpe*BA genes because they code for the apoprotein of PE and, as such, are integral to the genetic architecture of the spectral phenotype. We found that genes from strains which were similar based on 16s *r*DNA and *rpo*C1 did not necessarily have the same spectral phenotype, but that the PE genes from strains with the same spectral phenotype were always very closely related. This incongruence of phylogenies suggests strongly that the PE genes were acquired by lateral gene transfer between picocyanobacterial lineages. Topology of the trees for some clades do not allow us to rule out convergent evolution as an explanation for the similarity of sequence in PE genes from different lineages, but we have at least one concrete example of the acquisition of a new spectral phenotype in a lineage by lateral gene transfer.

IMPACT/APPLICATIONS

While it is relatively well known that strains in the same clade of picocyanobacteria may have different spectral phenotypes, the finding that strains with the same spectral phenotype have closely related PE genes is a new finding. This suggests the PE genes underlying each phenotype share common ancestry and that, once evolved, they have been readily incorporated into a number of lineages by lateral gene transfer. This means that they have been incorporated into many genetic backgrounds, lending the beneficial capacity to adapt to changing spectral environments to many different clades of picocyanobacteria with potentially different adaptations to other environmental variables.

RELATED PROJECTS

We have recently been funded to examine the role of invertebrate larvae in the midwater microbial loop by the National Science Foundation; this new project will utilize strains of picocyanobacteria isolated while we were conducting research on PE spectral signatures and benefits from our large data set on picocyanobacterial abundance and diversity in the Gulf of Mexico and in Florida coastal waters.

Experience with remote sensing and ocean color that the P.I. gained through this project has also led to affiliation with the Cooperative Institute for Ocean Satellite Studies (CIOSS) at Oregon State University and helped her obtain funding to develop optical tags for tracking harmful algal blooms - a collaboration with Dr. Pete Strutton at OSU funded by NOAA (www.coas.oregonstate.edu/habs; http://cioss.coas.oregonstate.edu/).

Additionally, because addition of a few flow cytometry samples to our run of Florida Bay samples at Bedford Inst. of Oceanography was relatively easy and inexpensive, we were able to collaborate with scientists on board the R/V Nathaniel B. Palmer and run samples they collected for us on the ICEFISH (International Collaborative Expedition to Collect and Study fish Indigenous to Sub-Antarctic Habitats) expedition in the austral winter of 2004 (http://www.icefish.neu.edu/). This series of samples provides a detailed look at the distribution of *Prochlorococcus*, PE-containing picocyanobacteria, heterotrophic bacteria, and small eukaryotes along a 2780 km S-N transect from Bouvetoya Island to Tristan da Cunha Island in the Atlantic Sector of the Southern Ocean. The transect crosses most major frontal features in the Antarctic Circumpolar Current. Dramatic changes were seen in the abundance of different taxa at each frontal boundary and the data showed that both PE-containing picoplankton and picoeukaryotes were part of the phytoplankton community in Antarctic Polar waters, even in winter.

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